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Spatial distribution pattern of Rhizobium *leguminosarum bv. vicieae* across legume growing areas of Tigray region

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ABSTRACT

Introduction: Several research findings stated that, faba bean had phenotypically diverse and symbiotically effective in Tigray, Ethiopia. However, limited researches were conducted in the region regarding biological nitrogen fixation with local varieties and races.

Objective: To address the limitations Spatial Distribution Pattern of Rhizobium *leguminosarum bv. Vicieae* across legume growing areas of Tigray region was investigated.

Methodology: A total of 112 root nodules with (107.9 average) nodules plant^{-1} were collected from 15 major faba bean growing districts of the region for isolation and characterization of indigenous root nodulating bacteria of the host plant. A serious of methods and procedures were undertaken to confirm the presumptive, eco-physiological and morphological characteristics of the isolated Rhizobium bacteria in triplicates.

Results: All the tested isolates were presumptively gram -ve, raised, translucent and white in color, failed to grow on peptone glucose agar, keto lactose test and Hofer's alkaline test. Phenotypically, 75% of the isolated candidates were appearing large mucoid and morphologically >80% of them were recorded more than 2mm colony diameter. Physiologically, 100 % of the isolates were grown well at a temperature range between 20°C and 30°C, pH values between 6 and 8, and in the range of 0.1 and 2.5% (w/v) NaCl concentrations. In addition to this, a faba bean nodulating bacterium uses a broad range (90-100%) of carbohydrate utilization. The effectiveness of the candidates confirms 65%, 18.33%, 15% and 1.6% were recorded as highly effective, effective, less effective and ineffective. Moreover, the highest and lowest symbiotic effectiveness was scored 457% and 21% respectively.

Conclusions: The study concluded that, the spatial distribution of faba bean nodulating bacteria across the region had diverse, eco-friendly, native and highly effective for agricultural sustainability.

Recommendations: Highly effective Rhizobial isolates should preserve for further research, field evaluation and extensive research should be conducted in the future.

Keywords: Eco-physiological; Phenotypic; Faba bean; Rhizobium; Symbiotic effectiveness; YEMA; Morphological; Colony; Nodulating bacteria.

1. Introduction

Interdependent N fixation in legume crops through special root nodulating bacteria known as rhizobia plays a vital role in naturally suitable agricultural systems. Nitrogen fixation is important especially in areas where legume crops limited access to synthetic or organic N fertilizers. Faba bean (*Vicia faba* L.) is one of the grain legumes that have the ability to grow over a wide range of soil and climatic situations of the Ethiopian highlands [1]. It occupies 28.45% of the total 1.6 million hectares of pulse cultivated and 3.53% of the total 12.6 million hectares of grain production [2]. The crop takes a lion share in the area under pulse production and serves as source of food and feed [3]. Nutritionally it contains 35% crude protein, around 50% of carbohydrates and no more than 15% is crude lipid [4]. Moreover, like other legume crops, this legume crop (*Vicia faba* L.) commonly integrated in different cropping systems in the highlands of Ethiopia as a sole crop or intercrop in crop rotation for its capability to fix nitrogen in symbiotic association with root nodule bacterial known as *Rhizobium leguminosarum vicia*. Literatures indicated that faba bean can fix atmospheric nitrogen up to 120 kg/ha/yr if suitable condition is available [5,6], and it can form mutualistic associations with various rhizobacterial species in Rhizobium genus, *Rhizobium leguminosarum* [7], *Rhizobium fabae* [8] and *Rhizobium laguerreae* [9]. Faba bean is an excellent legume crop that fixes atmospheric

nitrogen depends on sufficient effective rhizobia population [10,11]. Inoculation of grain legumes with active, sufficient and native nitrogen fixing bacteria (Rhizobia) prior to sowing has a significant role on legume yield production and affects soil microbial community in the rhizosphere [12]. According to [13] report, legume cultivated lands are estimated to fix 40-60 million tons of N annually in the globe. Recent researches also reported inoculation of grain legumes with effective bacterium can raise grain yield up to 500kg ha^{-1} Y^{-1} [13].

However, as its multifunctional benefit its production is low in the country, which is below the world's yield record (1700 kg ha^{-1}) [14-16]. This low yield production might be due to soil fertility depletion, soil acidity, disease occurrence, parasitic weed, lack of high yielding varieties [17,18] and low existence of indigenous nitrogen fixing bacteria in the Rhizosphere [19]. To solve this constraint, alleviation of soil fertility depletion through biological nitrogen fixation along with other agronomic practices is vital.

Some researchers show that, Ethiopia is considered as a secondary faba bean gene center [20,21], this indicates that the Ethiopian soils harbored diverse faba bean nodulating microsymbionts.

1.1. Study Objective

The main objective of the current research was targeted to develop environmentally competent, indigenous and accessible rhizobium bio-inoculants for faba bean growing areas of Tigray.

2. Methodology

2.1. Nodule collection

Pink and large nodules were collected during mid-flowering and late pod sating, while the sampling time is passing the required nodules were desiccate and rupture. Efficient nodules were collected from major faba bean growing districts of Tigray Region (Figure 1). Healthy plant roots were carefully removed from the soil using shovel and washed carefully using distilled water to remove the adhering materials away from the roots. The nodules were detached wisely from the roots using scissors and counted, and stored in screw-capped plastic tubes containing silica gel, with a cotton plug to separate nodules from the desiccant till the isolation undertake. Each plastic test tube was labeled using permanent marker. According to [22,23], the presence or absence of nodules was observed as mark that the soil had the ability of nodulating bacteria.

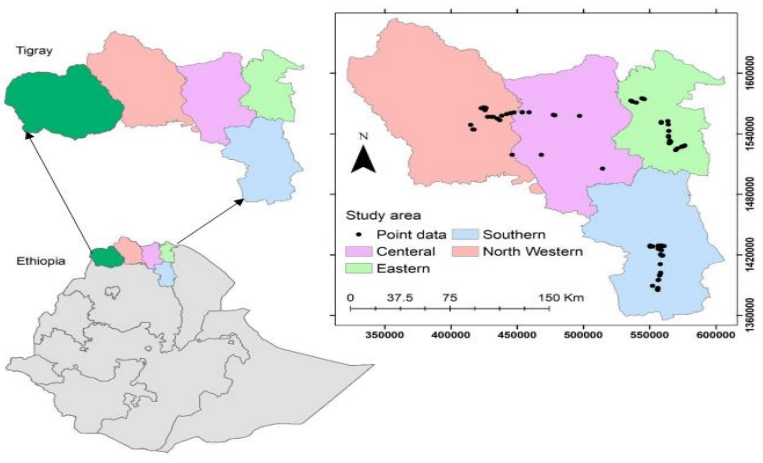

Figure 1. Map of the study area

2.2. Isolation and purification of faba bean root nodule bacteria

To evaluate the effective of the Rhizobial isolates, the collected nodules were washed frequently with tap water to remove the adhering soil particles from the nodule surface in petri-plates and followed by surface sterilized briefly with 70 % ethanol for 3 min and sodium hypochlorite solution for 30 min [23]. After washing several times in sterile deionized water, each nodule was crushed with the help of flamed glass rod in sterile 0.9% NaCl solution to obtain milky suspension under aseptic conditions. A loop full of the suspension were streaked on newly prepared yeast-extract-mannitol (YEM) agar medium, plate containing Congo red with pH 6.8 ± 0.2, and incubated at 28 ± 2 ^{0}C for 3-5 days. A total of the isolated (112) faba bean nodulating bacteria were purified and characterized on YMA. Identification of fast and slow growers was carried out using Gram's staining method. The shape, texture and color changes on YEM agar containing bromothymol blue were also observed. After incubation for another 3-5 days, single colonies were picked and purified by re-streaking on newly prepared YEMA plates and preserved temporarily at 4 ^{0}C on YEMA slants containing 0.3 % (w/v) $CaCO_3$ until further analysis [23]. Beside this, rhizobia isolates were permanently stored in YEM broth added with 20% of glycerol, at 20 ^{0}C.

2.3. Examining the presumptive properties

Each the isolated and preserved bacterium was examined in triplicates on yeast extract mannitol agar containing 10 g mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.1 NaCl, 1g Yeast, 0.025ml Congo red, 15g agar and 1000ml deionized water [22]. Gram staining to indicate the bacteria either gram positive or gram negative, peptone glucose agar (PGA), Keto lactose test, Hofer's alkaline test, and acid/base production was determined following the laboratory procedures [22,23].

2.4. In vitro eco-physiological and morphological characteristics

All in all, the isolated and purified faba bean Rhizobium isolates were further evaluated in vitro at different Eco-physiological (temperature, pH, salinity, carbohydrate and amino acid) properties and morphological characteristics following the recommended methods and procedures indicated in [22,23]. The growth performance and color change were recorded after 5-7 days of incubation period quantitatively either positive (+) or negative (-) indicating for the presence or absence of the bacterium.

2.5. In situ verification

Pure Rhizobial isolates were cultured in YEM broth at 28 ± 2 ^{0}C to mid-log phase (10^6 cells ml^{-1}), to verify the effectiveness or ineffectiveness of the collected isolates. Concentrated sulfuric acid (98%) treated and three times autoclaved (at 121 ^{o}C for 2 hrs) river sand was used as growing media to minimize the presence of nutrients. All the nominated isolates (112) were tested on surface sterilized [24] of the host plant (Faba bean), 5-10 surface sterilized faba bean seeds were placed separately in petri-dish on moist filter paper for germination at ideal temperature (27 ^{o}C). Five well germinated seeds were immersed in each rhizobial suspension for 8 hrs and planted to 1.5 kg capacity of sterilized river sand pot. The control checks were immersed in sterile water and the treatment was conducted in triplicates. The inoculated seedlings were incubated at Mekelle Research Center biotechnology greenhouse with a photoperiod of 12 hrs daylight at 25-27 ^{o}C, a night temperature of 20 ^{o}C, and 65% relative humidity. Including the N treated (positive control) and un-inoculated unfertilized (negative control) all pots were

supplied with quarter strength N-free nutrient solution at a rate of 100 ml pot^{-1} once a week and washed with deionized water as required to control salt accumulation. After 6 weeks of growing all plants were uprooted and washed carefully with tap water. The nodules were cut off from the plant roots to count and then dried at 70 ^{o}C for 24 hrs until constant weight. Numbers of nodules, Nodule volume, nodule dry weight, root dry weight and shoot dry weight per plant were recorded, and the symbiotic effectiveness was calculated using the following Equation 1,

$$\%SE = \frac{Inoculated\ SDW}{N\ fertilized\ SDW} X100 \qquad (1)$$

2.6. Data Analysis

The quantitative and qualitative data was completed using the average and performance range on the presence of growth (+) or absence of growth (-). The infectiveness of the isolates was determined for eco-physiological properties (temperature, pH and salinity) and morphological characteristics (texture, color, colony diameter and translucent). Amino acid and carbohydrate utilization percentage of the isolated strains was calculated and evaluated accordingly. Moreover, the agronomic parameters (shoot length, nodule number, nodule volume, nodule dry weight, shoot dry weight and root dry weight) were subjected to the analysis of variance (ANOVA) using SAS ver. 9.1 (2002) and Fisher's method for least significant different (LSD) at 5% hypothetical test.

3. Results

3.1. Isolation and presumptive characteristics

Prior to nodule collection to generate effective native Rhizobium isolates potential faba bean growing area were assessed and outlined. Accordingly, fifteen (15) districts were delineated and nominated as highly potential faba bean growing areas (Figure 1), the selected districts were geo referenced for further research. Though, a total of 112 faba bean plant roots with an average nodule number $plant^{-1}$ ranged from 23-186 were collected, transported to Mekelle Soil Microbiology laboratory and preserved at 4 ^{o}C refrigerator for isolation and characterization. The manifestation of nodules might be an indication of the potential and compatibility of Rhizobia present in the soil [25]. Necessary, most of the collected root nodules induced nodulation characteristics, implies that the legume growing areas had homologous native rhizobia. The occurrence of various average nodule number per plant (23-186 NN P^{-1}) from varied sampling sites was an indication of the rhizobia diversity in Tigray Soils. The collected root nodules were presumptively surface sterilized, isolated and preserved following soil microbiology laboratory standard procedures and methods. Off the collected root nodules 53.6% (60 isolates) were survived very well while the remain 46.4% (52 isolates) failed during biological test under laboratory test.

Table 1. Phenotypic growth profile of faba bean nodulating bacteria in-vitro

Phenotypic parameters						
Isolates	**Ave. NN**	**Texture**	**Transparency**	**Elevation**	**Colour**	**CDM (mm)**
EMFB	81-156	LM	T	R	White	>2
EAFB	23-186	LW	T	R	White	>2
OFB	67-184	LM	T	R	White	>2

KAFB	68-132	LM	T	R	White	>2
SSFB	89/115	LM	T	R	White	>2
GAFB	23-145	LM	T	R	White	>2
AWFB	48-114	LM	T	R	White	>2
TKFB	101/165	LM	T	R	White	>2
MZFB	63-124	LM	T	R	White	>2
TMFB	94-118	LM	T	R	White	>2
LMFB	102/109	LW	T	R	White	>2
ADFB	86	LM	T	R	White	>2
HSFB	141	LW	T	R	White	>2

Where: Ave.NN= average nodule number, LM =large mucoid, LW =large watery, T =translucent, R= raised, CDM =colony diameter.

3.2. Eco-physiological and morphological characteristics of Faba bean nodulating bacteria

The confirmatory test of the isolated rhizobia strains for eco-physiological and morphological characteristics were used to confirm the indigenous rhizobial species to their eco-physiological and morphological adaptability to different condition in vitro. Hence, the confirmatory result revealed that 53.6% (60) of the isolates were confirmed to be the species of faba bean nodulating bacteria called rhizobia, this is an indication of root nodule bacteria (rhizobia) characteristics [22,23,31,32,33]. Phenotypically, all the screened isolates were transparency, raised shape and white in color which is the characteristics of rhizobia [23]. As indicated in table 1, 75% of the screened rhizobia isolates were identified as large mucoid, and 80% recorded > 2mm colony diameter, similarly, 85% of rhizobial isolates of field pea from southern Tigray was recorded as large mucoid [28]. Regarding to morphological parameters, all the rhizobium isolates were failed to grow on peptone glucose agar, keto lactose, Hofer's alkaline test, color less on YEMA supplemented with Congo red (Table 2). This indicates that, the preliminary confirmation of non-contaminant gram-negative rhizobia. More than 75% of the identified isolates were recorded as yellowish in color and the remain 25% isolates (FB-3, 8, 17, 19, 22, 28, 35, 41, 45, 60, 63, 98, 104, 108 and 112) changed the YEMA-BTB medium in to Blue (Table 2) which is the characteristics of acid base production.

Table 2. Morphological growth characteristics of faba bean nodulating bacteria

Morphological characteristics						
Isolates	**YEMA-CR**	**BTB**	**PGA**	**KLT**	**HAT**	**Gram stain**
EMFB (5)	Colourless	Yellow/Blue	No growth	No	No	Negative
OFB (7)	Colourless	Yellow/Blue	No	No	No	Negative
EAFB (18)	Colourless	Yellow/Blue	No	No	No	Negative

KAFB (4)	Colourless	Yellow	No	No	No	Negative
SSFB (2)	Colourless	Yellow	No	No	No	Negative
GAFB (7)	Colourless	Yellow/Blue	No	No	No	Negative
AWFB (5)	Colourless	Yellow	No	No	No	Negative
TKFB (2)	Colourless	Yellow	No	No	No	Negative
MZFB (3)	Colourless	Yellow/Blue	No	No	No	Negative
TMFB (3)	Colourless	Yellow/Blue	No	No	No	Negative
LMFB (2)	Colourless	Yellow/Blue	No	No	No	Negative
ADFB (1)	Colourless	Yellow	No	No	No	Negative
HSFB (1)	Colourless	Blue	No	No	No	Negative

Where: BTB= bromothymol blue, PGA = Peptone Growth Agar, KLT = Keto lactose test, HAT = Hofer's alkaline test, No = no-growth.

3.3. Verification and evaluation of symbiotic effectiveness of faba bean nodulating bacteria on sand culture

For validating the symbiotic effectiveness and ineffectiveness of rhizobia nodulating bacteria authentication under sand culture is among the prerequisite characteristics of locally isolated bacterial strains [38]. After the biochemical test, only sixty (60) isolates were permitted the nodulation characteristics and authenticated as *Rhizobium leguminosarum bv. Viciae* (Table 3). Off the collected (112 isolates) the remaining 60 (50%) isolates, which were not induced nodules on faba bean root might be contaminants or rhizobia of other plants [35]. The specific *Rhizobium* genotypes that form nodules obtained from cross-inoculation groups may not effective faba bean nodules [39]. Out of the collected isolates 65%, 18.33%, 15% and 1.6% were recorded as highly effective, effective, less effective and ineffective (Table 3) respectively. Rhizobial isolates TMFB-103, LMFB-110 and EAFB-38 scored the highest symbiotic effectiveness of 457%, 347% and 241% respectively. The highest and lowest percentage symbiotic effectiveness were obtained from Tahitai-machew and Laelai-maichew districts respectively, this indicates the potential of bacterial diversity in the study area. The highest nodule number (76) plant^{-1} were recorded from isolate EAFB-8 which is generated from Emba-alaje district followed by GAFB-45 (55 NN/P) and the lowest nodule number were obtained from EAFB-33 (5NN/p) followed by EMFB-21 (7NN/p) (Table 3) respectively. The current result is much smaller than results obtained by [36] rhizobial isolates from central Ethiopia and recorded highest nodule number in the range of 186.7-172. The next prerequisite to confirm the effectiveness of the isolated rhizobacteria is nodule volume, though, the highest nodule volume was obtained from EAFB-8 (3.6) followed by EAFB-45 (3.25), and the lowest nodule volume were recorded from KAFB-81 (0.5) followed by EAFB-35 (0.63) respectively. A wide range of nodule and shoot dry weight was recorded from the study areas, which is in the range of 100-850mgplant^{-1} nodule dry weight and 0.87-4.96gmplant^{-1} of shoot dry weight, with symbiotic effectiveness of 81-203% and 43% -172%. Similarly, [37] reported that, field isolates were recorded nodule and shoot dry weight in the range of 104-121mgplant^{-1} and 2.3-2.9gmplant^{-1} respectively. The

highest nodule and shoot dry weight were obtained from ADFB-111(850mg) and EAFB-44 (4.96gm) and the lowest dry weight was recorded from EAFB-39 (100mg) and MZFB-98 (0.87gm) respectively. [36] Reported that, highest nodule dry weight (0.263gm plant^{-1}) was obtained from similar cultivar. Interestingly, the collected isolates were symbiotically significant effectiveness on the noted districts as listed in Table 3. Then after, majority of bacterial isolates (18) were obtained from Emba-alaje district, this indicates might be due to the potential suitability of faba bean growing district. Off those 12 (66.6%) bacterial isolates were recorded as symbiotically highly effective (81-241%) and the remain 6 (33.3%) isolates were less effective (34-47%), none of the isolates scored ineffective (<30%) (Table3). Seven isolates were emerged from Ofla and Ganta-Afeshum districts and permits the verification and evaluation characteristics under controlled environment (acid treated sand culture), consequently, five isolates (71.42%) from each district categorized under highly effective (>80%SE) and the remaining two isolates (28.57%) from the notified districts were effective (OFB-19) and less effective (OFB-17, GAFB-60 and GAFB-63). Enda-mohoni also owned five isolates (EMFB-2, EMFB-3, EMFB-4, EMFB-5 and EMFB-21), and four (80%) of them were scored symbiotically highly effective (109-225%). Kilte-Awlaelo and Atsibi-Wenberta districts from Eastern zone of Tigray region was aseptically generates four rhizobial isolates each (Table 3 and Figure 7). Hence, 3 (75%) of the isolates from Kilte-Awlaelo score highly effective (86-189%SE), while the 3 (75%) isolates from Atsibi-Wenbeta were scored effective (69-73%SE). Tahitay-Maichew and Medebai-zana each district originates 3 (three) rhizobial isolates, two isolates from Lae'lai-Maichew and Tahtay-Koraro and only one isolates was obtained from Adwa, Asgede-thimbla and Hagere-selam (Table 3, Figure 7, 8, 9 and 10). Surprisingly, the highest percentage symbiotic effectiveness (457%) and ineffective (21%) isolates were obtained from Central zone Tahitay-Maichew and Lae'lai-Maichew) respectively (Table 3 & Figure 9).

Table 3. Invitro Verification of Rhizobial Isolates on sand culture

Isolates	Shoot length at 50% flowering(cm)	Nodule number after 45 days NN/P	Nodule volume NV (L)	Nodule dry weight (mg)	Shoot dry weight (g)	Root dry weight (g)	%Symbiotic effectiveness	Effectiveness	District	Soil pH
EMFB-2	41.33^{ab}	9.00^{t-x}	0.75^{k-n}	100^{ef}	2.97^{abc}	2.29^{a-e}	116	HE	Enda-mohoni	7.120
EMFB-3	40.50^{ab}	43.00^{c-i}	2.65^{a-f}	280^{c-f}	2.33^{abc}	2.87^{abc}	79	E	Enda-mohoni	6.890
EMFB-4	43.00^{ab}	54.00^{bcd}	2.75^{a-e}	200^{def}	3.26^{abc}	2.47^{a-e}	182	HE	Enda-mohoni	7.050
EMFB-5	45.25^{ab}	40.50^{c-k}	3.10^{abc}	150^{def}	3.53^{abc}	2.12^{a-e}	109	HE	Enda-mohoni	7.450
EAFB-7	48.17^{ab}	41.00^{c-j}	0.75^{k-n}	230^{def}	3.74^{abc}	2.24^{a-e}	141	HE	Emba-alaje	7.300
EAFB-8	41.33^{ab}	76.00^{a}	3.60^{a}	600^{ab}	3.28^{abc}	1.98^{a-e}	34	LE	Emba-alaje	7.790

OFB-10	43.58^{ab}	45.00^{b-h}	2.25^{b-i}	400^{bcd}	3.44^{abc}	2.07^{a-e}	169	HE	Ofla	7.810
OFB-13	42.33^{ab}	16.50^{o-w}	2.00^{c-j}	200^{def}	3.92^{abc}	2.99^{abc}	211	HE	Ofla	7.090
OFB-14	39.50^{ab}	23.00^{l-u}	2.60^{a-g}	280^{c-f}	2.64^{abc}	1.81^{a-f}	82	HE	Ofla	6.830
OFB-15	46.17^{ab}	17.00^{o-w}	0.75^{k-n}	150^{def}	3.61^{abc}	2.67^{a-d}	86	HE	Ofla	6.990
OFB-17	34.33^{ab}	48.50^{b-e}	2.65^{a-f}	180^{def}	2.74^{abc}	1.74^{b-f}	32	LE	Ofla	7.290
OFB-18	43.67^{ab}	45.00^{b-h}	3.05^{abc}	300^{cde}	3.93^{abc}	1.78^{b-f}	196	HE	Ofla	7.470
OFB-19	43.83^{ab}	42.50^{c-i}	2.10^{c-j}	200^{def}	3.51^{abc}	1.68^{b-f}	72	E	Ofla	ND
EMFB-21	49.00^{a}	7.00^{u-x}	1.10^{j-n}	300^{cde}	3.28^{abc}	2.37^{a-e}	225	HE	Enda-mohoni	7.450
EAFB-22	39.83^{ab}	27.50^{i-r}	1.75^{d-k}	180^{def}	3.10^{abc}	1.95^{a-e}	71	E	Emba-alaje	7.180
EAFB-24	43.67^{ab}	25.50^{j-r}	3.00^{abc}	160^{def}	3.67^{abc}	2.24^{a-e}	100	HE	Emba-alaje	7.460
EAFB-26	46.00^{ab}	22.50^{l-u}	1.37^{i-m}	300^{cde}	2.79^{abc}	1.91^{a-e}	146	HE	Emba-alaje	7.400
EAFB-28	37.00^{ab}	40.00^{c-k}	2.65^{a-f}	150^{def}	2.81^{abc}	2.17^{a-e}	39	LE	Emba-alaje	7.570
EAFB-30	38.00^{ab}	38.00^{d-l}	2.85^{a-d}	400^{bcd}	2.34^{abc}	1.58^{b-f}	223	HE	Emba-alaje	7.680
EAFB-32	41.00^{ab}	20.00^{n-v}	1.50^{g-m}	150^{def}	2.24^{abc}	1.89^{a-e}	85	HE	Emba-alaje	7.790
EAFB-33	47.17^{ab}	5.00^{vwx}	0.75^{k-n}	100^{ef}	3.15^{abc}	2.18^{a-e}	82	HE	Emba-alaje	7.160
EAFB-34	42.50^{ab}	28.00^{i-q}	1.75^{d-k}	160^{def}	2.98^{abc}	2.35^{a-e}	172	HE	Emba-alaje	7.050
EAFB-35	45.50^{ab}	29.00^{h-p}	0.63^{lmn}	160^{def}	2.51^{abc}	1.78^{b-f}	47	LE	Emba-alaje	7.200
EAFB-38	43.33^{ab}	28.00^{i-q}	1.75^{d-k}	220^{def}	2.49^{abc}	1.46^{b-f}	241	HE	Emba-alaje	7.720
EAFB-39	44.83^{ab}	12.50^{p-x}	0.75^{k-n}	100^{ef}	3.21^{abc}	2.58^{a-e}	81	HE	Emba-alaje	7.370
EAFB-41	41.17^{ab}	31.50^{f-o}	1.60^{f-m}	400^{bcd}	3.44^{abc}	2.89^{abc}	66	E	Emba-alaje	7.340
EAFB-42	39.00^{ab}	7.50^{u-x}	1.10^{j-n}	550^{bc}	2.47^{abc}	1.71^{b-f}	130	HE	Emba-alaje	7.750
EAFB-43	43.50^{ab}	28.50^{i-q}	2.00^{c-j}	150^{def}	3.76^{abc}	1.67^{b-f}	95	HE	Emba-alaje	7.350
EAFB-44	52.00^{a}	46.00^{b-f}	2.50^{a-h}	250^{def}	4.96^{a}	2.37^{a-e}	172	HE	Emba-alaje	7.100
EAFB-45	45.33^{ab}	59.50^{b}	3.25^{ab}	310^{b-d}	2.84^{abc}	2.01^{a-e}	50	LE	Emba-alaje	ND
KAFB-48	42.08^{ab}	22.50^{l-u}	1.50^{g-m}	200^{def}	3.11^{abc}	1.90^{a-e}	72	E	Kilte-awla'elo	8.050

KAFB-49	42.00^{ab}	37.50^{e-l}	1.50^{g-m}	300^{cde}	3.65^{abc}	1.23^{c-f}	189	HE	Kilte-awla'elo	7.620
KAFB-50	44.17^{ab}	24.50^{k-t}	0.75^{k-n}	550^{bc}	2.41^{abc}	2.56^{a-e}	86	HE	Kilte-awla'elo	7.190
KAFB-51	38.50^{ab}	19.50^{n-v}	1.25^{i-m}	110^{def}	2.82^{abc}	1.99^{a-e}	143	HE	Kilte-awla'elo	6.940
SSFB-53	48.33^{ab}	10.50^{s-x}	0.75^{k-n}	120^{def}	1.29^{bc}	2.64^{a-d}	64	E	Sae'sie-tsaedaemba	7.280
SSFB-54	39.17^{ab}	43.00^{c-i}	1.65^{e-l}	250^{def}	3.74^{abc}	2.36^{a-e}	111	HE	Sae'sie-tsaedaemba	6.620
GAFB-59	43.83^{ab}	28.00^{i-q}	1.75^{d-k}	200^{def}	3.70^{abc}	2.18^{a-e}	151	HE	Ganta-afeshum	ND
GAFB-60	44.08^{ab}	20.00^{n-v}	1.00^{j-n}	150^{def}	2.82^{abc}	3.09^{ab}	50	LE	Ganta-afeshum	6.950
GAFB-62	35.25^{ab}	31.00^{g-o}	1.65^{e-l}	150^{def}	2.09^{abc}	0.92^{def}	159	HE	Ganta-afeshum	6.680
GAFB-63	37.50^{ab}	35.50^{e-n}	2.75^{a-e}	160^{def}	3.42^{abc}	2.31^{a-e}	48	LE	Ganta-afeshum	7.110
GAFB-64	47.50^{ab}	43.50^{b-i}	1.75^{d-k}	310^{b-e}	4.44^{ab}	3.63^{a}	177	HE	Ganta-afeshum	ND
GAFB-66	40.33^{ab}	47.50^{b-f}	2.55^{a-g}	280^{c-f}	3.04^{abc}	2.27^{a-e}	88	HE	Ganta-afeshum	7.100
GAFB-70	44.00^{ab}	55.00^{bc}	2.75^{a-e}	350^{b-e}	4.48^{ab}	0.79^{ef}	161	HE	Ganta-afeshum	7.110
AWFB-75	43.33^{ab}	50.50^{b-e}	2.50^{a-h}	300^{cde}	3.04^{abc}	2.89^{abc}	73	E	Atsbi-wenberta	7.660
AWFB-77	48.17^{ab}	43.50^{b-i}	1.60^{f-m}	260^{c-f}	2.68^{abc}	2.49^{a-e}	74	E	Atsbi-wenberta	6.800
AWFB-78	41.67^{ab}	37.50^{e-l}	1.50^{g-m}	250^{def}	2.82^{abc}	0.85^{def}	107	HE	Atsbi-wenberta	6.750
AWFB-81	41.67^{ab}	2.50^{wx}	0.50^{mn}	200^{def}	3.15^{abc}	1.97^{a-e}	69	E	Atsbi-wenberta	7.490
AWFB-82	40.33^{ab}	31.00^{g-o}	1.50^{g-m}	160^{def}	2.56^{abc}	2.57^{a-e}	159	HE	Asgede-tsimbla	6.190
TKFB-93	30.00^{ab}	48.00^{b-d}	2.25^{b-i}	300^{cde}	2.29^{abc}	0.77^{ef}	89	HE	Tahitai-koraro	6.740
TKFB-96	33.83^{ab}	20.50^{n-v}	1.55^{f-m}	160^{def}	2.87^{abc}	2.15^{a-e}	102	HE	Tahitai-koraro	6.650
MZFB-97	36.33^{ab}	14.00^{p-x}	1.10^{j-n}	300^{cde}	2.53^{abc}	2.24^{a-e}	87	HE	Medebai-zana	6.510
MZFB-98	26.25^{ab}	11.00^{s-x}	1.00^{j-n}	300^{cde}	0.87^{c}	2.27^{a-e}	43	LE	Medebai-zana	6.630
MZFB-99	32.50^{ab}	20.50^{n-v}	1.25^{i-m}	170^{def}	3.28^{abc}	1.33^{b-f}	77	E	Medebai-zana	6.700
TMFB-102	40.17^{ab}	31.50^{f-o}	1.50^{g-m}	150^{def}	2.69^{abc}	1.99^{a-e}	86	HE	Tahitai-maichew	6.690
TMFB-103	37.83^{ab}	19.00^{o-v}	1.00^{j-n}	150^{def}	3.12^{abc}	2.59^{a-e}	457	HE	Tahitai-maichew	6.700

TMFB-104	29.42^{ab}	23.00^{l-u}	1.00^{j-n}	200^{def}	2.71^{abc}	1.66^{b-f}	69	E	Tahitai-maichew	6.890
LMFB-108	36.42^{ab}	21.50^{m-u}	0.75^{k-n}	150^{def}	2.39^{abc}	1.75^{b-f}	21	IE	Lae'lai-maichew	5.990
LMFB-110	37.00^{ab}	11.50^{r-x}	1.10^{j-n}	150^{def}	2.32^{abc}	2.55^{a-e}	374	HE	Lae'lai-maichew	5.640
ADFB-111	36.33^{ab}	29.00^{h-p}	1.40^{h-m}	850^{a}	3.20^{abc}	1.79^{b-f}	203	HE	Adwa	6.010
HSFB-112	34.00^{ab}	21.00^{n-v}	0.75^{k-n}	150^{def}	1.95^{abc}	2.27^{a-e}	49	LE	Hagere-selam	5.940
N-	34.00^{ab}	0.00^{x}	0.00^{n}	0.00^{f}	1.73^{abc}	2.20^{a-e}				
N+	36.67^{ab}	0.00^{x}	0.00^{n}	0.00^{f}	1.37^{abc}	2.37^{a-e}				
CV	27.82	28.74	34.00	63.17	59.78	44.97				
LSD	22.65	16.49	1.12	0.29	3.49	1.83				
P value	0.9998	<.0001	<.0001	0.0129	1.000	0.4555				

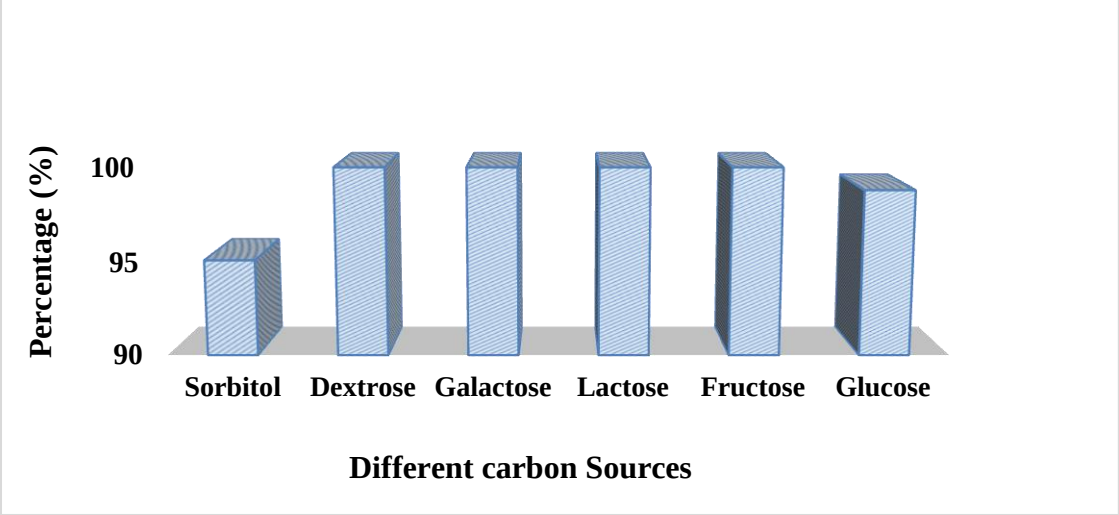

Figure 2. Percentage carbon source utilization by faba bean Rhizobium isolates

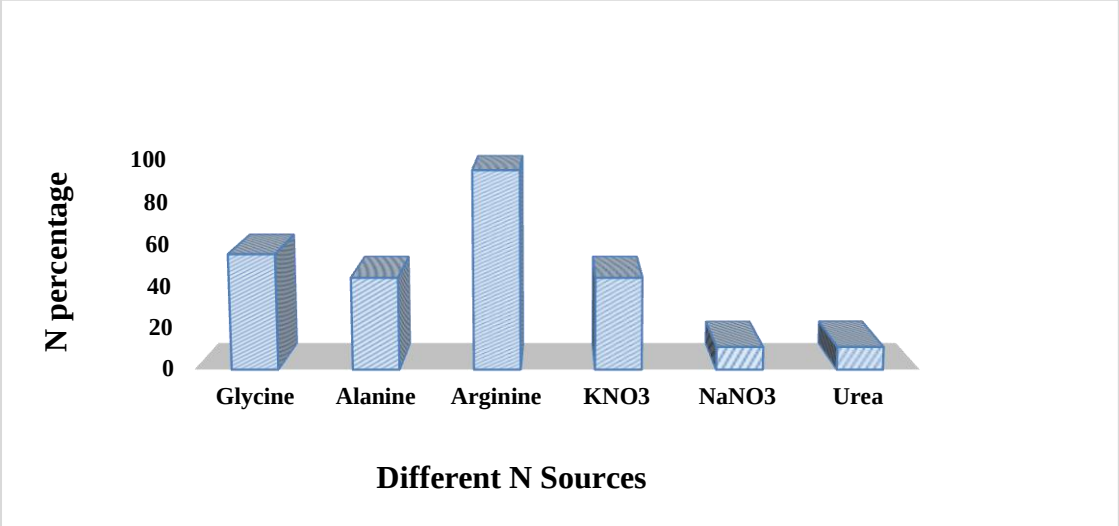

Figure 3. Various amino acid percentage utilization by faba bean Rhizobium strains

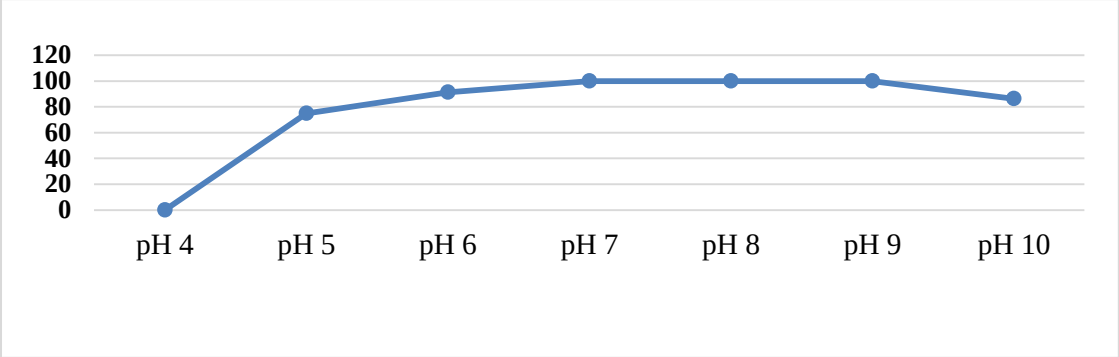

Figure 4. pH Sensitivity sketching pattern analysis

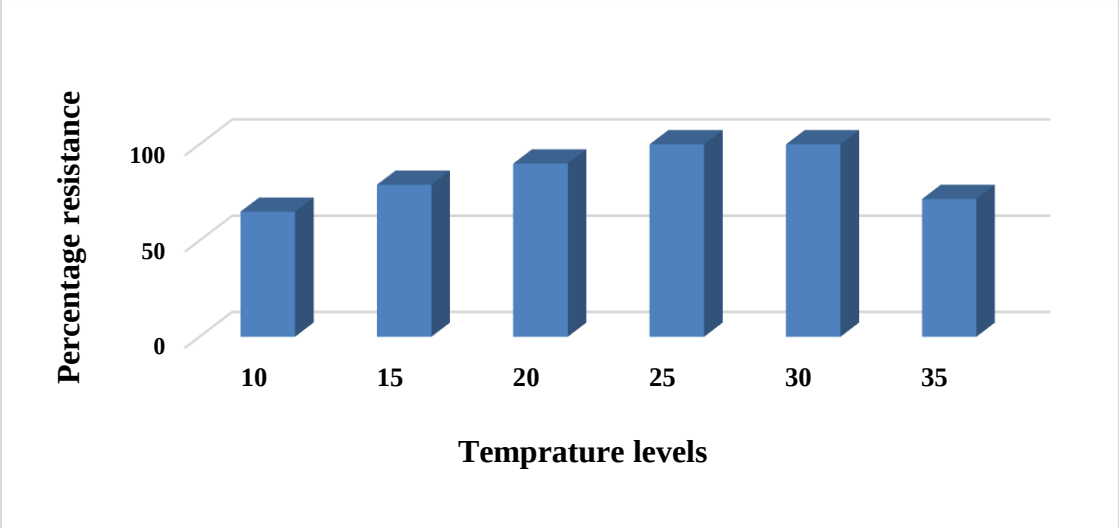

Figure 5. Different temperature sensitivity resistance of the candidate Rhizobial isolates

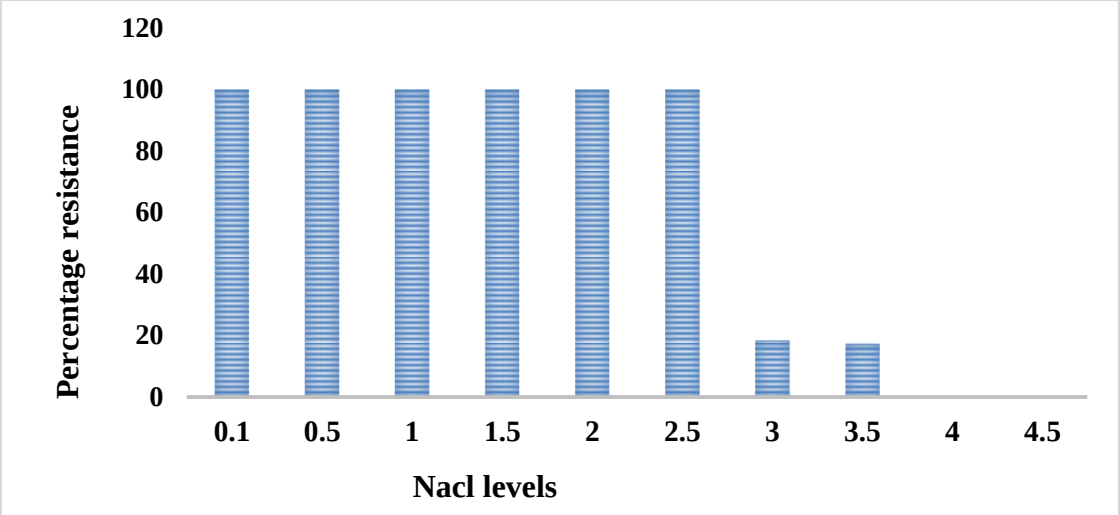

Figure 6. Fitness of faba bean nodulating bacteria to various NaCl concentrations under controlled environment (laboratory)

3.4. Eco-physiological characteristics of the isolated faba nodulating bacteria

Six carbohydrate and amino acid sources were permitted in triplicates for the approval of the isolated candidates under laboratory condition. Accordingly, 100% of the isolates were utilized Dextrose, Galactose, Lactose; Fructose

as carbon sources and Arginine nitrogen sources (Figure 3 and 4). As figure 3 shows that, Urea and $NaNO_3$ has recorded similar utilization (11.1%) by the rhizobial isolates. Various levels of temperature, pH and sodium chloride concentration sensitivity resistance were evaluated following the standard procedures, concerning this all of the isolates were resist pH starting from 6 to 9, 25 to 30^{O}c temperature and 0.1 to 2.5% Nacl concentration (Figure 4, 5 and 6). Percentage of resistance to sodium chloride concentration of the nominated isolates were decreased beyond 3% (w/v) and none of the isolates were survived at pH 4% and 4.5% Nacl concentration (Figure 4 and 6) respectively.

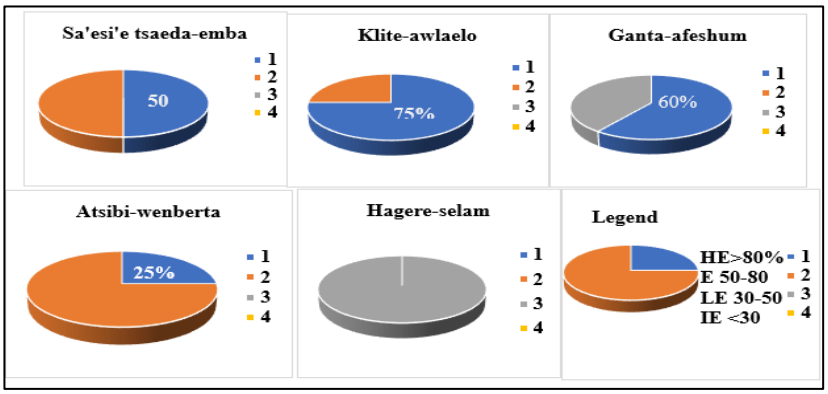

Figure 7. Effectiveness of Faba bean nodulating bacteria originated from Eastern zone of Tigray Region. Two isolates from Sa'esi'e tsaeda-emba 50% highly effective, four isolates from Klite-awlaelo 75% highly effective, seven isolates from Ganta-afeshum 60% highly effective, four isolates from Atsibi-wenberat 25% highly effective and one from Hagere-selam 100% less effective

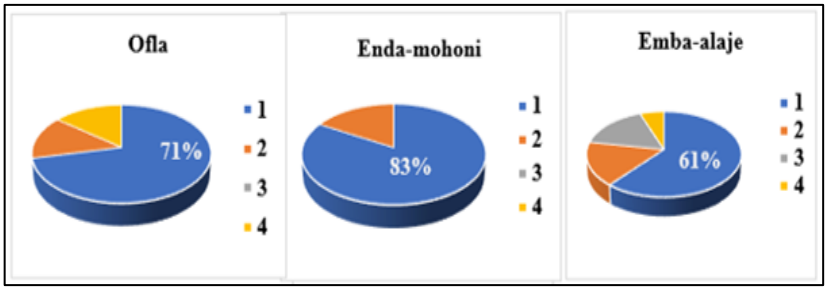

Figure 8. Effectiveness of Faba bean nodulating bacteria on sand culture originated from Southern Tigray. Seven bacterial strains from Ofla 71% highly effective, five isolates from Enda-mohoni 83% highly effective and 18 isolates from Emba-alaje 61% highly effective

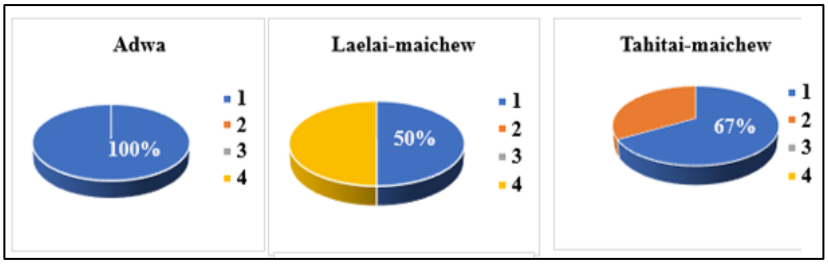

Figure 9. Effectiveness of Faba bean nodulating bacteria isolated from Central Zone of Tigray. Only one isolate was obtained from Adwa and 100% is highly effective, two isolates from Laelai-maichew 50% of them highly effective and three isolates from Tahitai-maichew and 67% highly effective

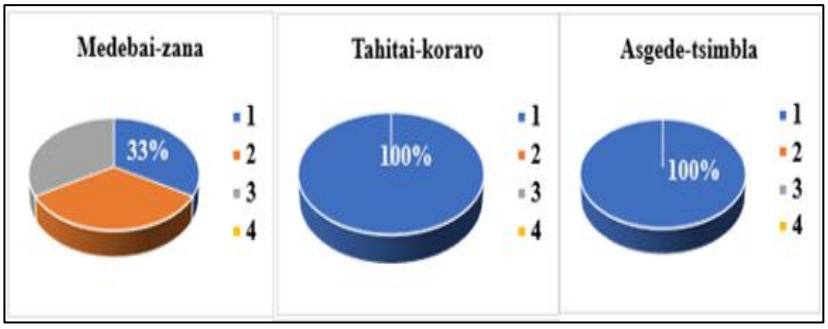

Figure 10. Symbiotic effectiveness of faba bean nodulating bacteria isolated from North western zone of Tigray. Medebai-zana owned three isolates and 33% highly effective, two isolates from Tahitai-koraro scored 100% highly effective and only one strain obtained from Asgede-tsimbla and 100% highly effective (>80%SE)

4. Discussions

Nodule formation and nitrogen fixation by a particular plant occurs only in the presence of well-matched rhizobia in the soil rhizosphere. Subsequently, the quantity and mass of nodules attained in this research achieves the nodulation potential and compatibility of rhizobia originated in the soil to form symbiotic association with the host plant (Faba bean). [26] Confirms this research due to the presence of rhizobia in Tigray soils that form symbiotic association to the host legume plants. [27] Has obtained 46 rhizobial isolates of faba bean nodulating bacteria from Tahtay Koraro district.

Similarly, [28] reported 80 bacterial isolates of field pea (*Pisum sativum var. abyssinicum*) nodule forming bacteria were originated from southern Tigray, [29] obtained 108 bacterial isolates of faba bean from faba bean growing areas of central and southern Ethiopia. The formation of ample number of nodules on legume plants in any soil depends on the existence of high quantity of homologous rhizobia in the soil [30]. The conformity of large mucus production by the eco-friendly rhizobium isolates in this research implies the superiority of the bacteria allied with the survival will be the fittest. Rhizobial isolates having high mucus production ability are highly competitive advantage in root hair infection, colonization, and nodule formation [34]. A wide range of faba bean nodulating bacteria isolates were recorded as symbiotically highly effective (81-457%), this implies that, the abundance of symbiotically effective native rhizobial isolates existed in the study area.

According to [13] report legumes can meet their N demand through the act of biological nitrogen fixation, though the current research fulfills these characteristics. The amount of N fixed by the host plant depends on crop type, soil type and availability of nitrogen in the soil, soils with low nitrogen has the ability to fix more N from the atmosphere [13]. According to the nitrogen reference manual report [13] faba bean producers of the indicated district fulfills their N requirement through the inoculation of the highly effective isolates developed from that district.

5. Conclusions and Recommendations

The presence and spatial distribution of faba bean nodulating bacteria from various soils were the most predominant request for their effectiveness on the host plant. Though, identification and determination of native and effective Rhizobacteria is a vital role in understanding the distribution and diversity of the Rhizobia in the study area. The current research noted that, Tigray soils harbored various Rhizobial strains which are phenotypically diverse and

morphologically effective. Majority of the isolated strains were symbiotically highly effective, and more resistance to various pH (4-10), temperature (10-35°C), and salt concentration (0.1 to 4.5% (W/V)), this is an indication of the capability of the bacterial strains that fulfill the N requirement of the host plant.

The authors recommended that, (1) the highly effective Rhizobial isolates should well preserve for further research, (2) field evaluation should be conducted, (3) should test for similar agro-ecology and soil type, and (4) extensive research on isolation and characterization is important for legume yield production.

Declarations

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Competing Interests Statement

The authors declare having no competing interest with any party concerned during this publication.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors made full contribution starting from proposal writing, visualization, methodology, data analysis, first draft writing, review and editing.

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